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Different types of spectroscopic techniques (steady state and pulsed laser spectroscopy, visible and near-IR Raman spectroscopy, UV fluorescence and excitation spectroscopy, time-resolved spectroscopic kinetics, and light scattering and propagation) were used to investigate cancerous and normal human tissues from different organs (breast, uterus, cervix, ovary, kidney, lung, prostate, and bladder) without injection of any dyes and chemicals and cultured cells (breast, lung and kidney cells). Most of our effort concentrated on breast tissues which have been distinguished by our new spectroscopic technology. More than 17 publications have been achieved over the past three years in both an understanding of the optical and spectroscopic characteristics of cancerous and normal tissues and instrumentation development by our biomedical physics and engineering groups.

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FINAL TECHNICAL REPORT

Cancer Diagnosis by Laser Spectroscopy

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Contract Number:	N0014-87-K-0431
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Principal Investigator:	R. R. Alfano
Affiliation:	The City College of New York
	Institute for Ultrafast Spectroscopy and Lasers
Researchers:	G. C. Tang, A. Pradhan, W. Sha Glassman ,
	B. B. Das, and C. H. Liu, K. M. Yoo
Research Foundation:	#447240
Report date:	January 31, 1991

Table of Contents

I. Introduction

The objective of this project was to use optical spectroscopy to detect cancer. Over the years, hematoporphyrin derivatives (HPD) have been employed as fluorescent markers for cancer diagnosis. However, these extrinsic fluorescent dyes present themselves as foreign agent, and have been known to interact with the normal cellular environment. There is a need to develop a new optical technique without the use of dyes or any chemicals to detect pathological changes in malignant parts of tissues without interfering with the normal surroundings.

There are several kinds of intrinsic fluorescent molecules present in tissues. Over past three years, we investigated various kinds of cancerous and normal human tissues from different organs without injection of any dyes and chemicals using different kinds of spectroscopic techniques. The tissues which were investigated included breast, uterus, cervix, ovary, kidney, lung, prostate, and bladder. Also, cultured breast cells were investigated. We concentrated on breast tissues for most of our work becuase of close relationship with Doctors Hospital.

Many modern spectroscopic techniques we have used are steady state laser spectroscopy, laser pulsed spectroscopy, visible and near-IR

Raman spectroscopy, UV spectroscopy, excitation spectroscopy, time-resolved spectroscopic kinetics, and light scattering and propagation.

Over the past three years, significant results with 17 publications have been achieved in both an understanding of the optical and spectroscopic characteristics of cancerous and normal tissues and the technique development by our biomedical physics and engineering groups.

We believe that this project is at the beginning stages and needs to be continued to enter into clinical testing.

II. A Summary of Research

The following highlights our accomplishments:

(1) UV Fluorescence Spectroscopy

To develop a diagnostic method for cancer detection it is essential to separate malignant tumors not only from normal tissues but from benign tissues and tumors. This seems possible from the work of B.Das and W.Sha Glassman using ultraviolet fluorescence spectroscopy. This is a major breakthrough, in particular, with excitation wavelengths from 270 to 315 nm.

Over sixty samples of cancerous and non-cancerous breast tissues were tested using UV fluorescence spectroscopic methods. Each sample was excited by the UV light source at 300 nm and the fluorescence spectra were measured from 320 nm to 580 nm using a lamp based fluorescence spectrometer (Perkin-Elmer LS50). The ratio of fluorescence intensities at 340 nm to that at 440 nm was found to be different between malignant tumor, benign tumor , benign tissue, and normal tissue. This pair of wavelengths (340 and 440 nm) was selected to minimize the effect of self-absorption on fluorescence by blood in the tissues because the blood absorption coefficients are almost the same for the both wavelengths. The average ratio for nineteen malignant samples is 15.7, for twenty benign tissues and (fibroadenoma) tumors is 4.7. The ratio range for cancer extends from 10 to 30 while that for benign samples is 2 to 9. Pathological data of each sample were known before the experiments performed and the results were used to build up a ratio histogram. Similar results were obtained for GYN tissues with small number of sample set (see section 3).

This information will be incorporate into the unit to be used for clinical studies and ratiometer which was funded by Mediscience Technology Corp. (MTC).

Later several blind samples were tested and the results were in 98% agreement with the diagnosis made by the pathologists. This method of detection not only separates malignant from benign samples but provides a simple technique to be used. Work is needed to determine fluorophors causing the fingerprint of cancer such as tryptophan, tyrosine and NADPH.

(2) Near Infrared Raman scattering spectroscopy

Fluorescence from tissues tends to overwhelm the visible Raman spectra. It is well known that near-infrared excitation coupled with Fourier-Transform Raman scattering from materials can efficiently eliminate fluorescence background which covers the visible and UV spectral regions.

The near infrared Raman scattering spectra from benign breast tissues, benign breast tumors and malignant breast tumors have been measured using a Quantronix model 114 Nd:YAG CW laser at 1064 nm of about 1 watt power to suppress the affect of fluorescence. This work was headed by Ms. Liu.

The differences in Raman scattering spectra were obtained over Raman frequency shifts from 1000 to 1800 cm^{-1}. The intense Raman

lines are located at 1078, 1300, 1445 and 1651 cm^{-1} for benign tissue; and 1240, 1445,and 1659 cm^{-1} for benign tumor; and 1445 and 1651 cm^{-1} for malignant tumor. The lack of lines from 1000 to 1300 cm^{-1} for malignant tumors offers a diagnostic approach. The intensities and numbers of Raman lines may be used to set up diagnosing criteria.

In the limited number of samples, we have distinguished between benign and malignant tumors using IR Raman spectral positions, number of lines and intensities.

(3) Investigation of gynecological tract cancer

The fluorescence and excitation spectra were measured by Ms. Sha Glassman from different tissues of gynecological tracts: uterus, cervix, and ovary using laser and lamp excitation. Experiments were performed using different excitation wavelength on fresh tissues, dried tissues, and also the interstitial medium. Significant differences have been found between the normal and tumor tissues. Statistics analysis are given to show the specificity, sensitivity, and accuracy for determining malignancy by these spectroscopic differences using ratios at key wavelengths of 340 and 440 nm. All the cancer tumor's ratio values are above 11, and all the normal tissue's ratio values are below 10. The

separation of cancer and normal tissues is fairly clear. Only a limited results of samples were studied. The reasons for spectra profile differences may be attributed to the transformation of the local distribution of the different molecular groups such as flavins, tryptophan, tyrosine, hemoglobin, etc.

(4) Time-resolved Fluorescence Kinetics from Benign and Malignant Tumors

Time-resolved fluorescence kinetics from benign and cancer tissues have been measured by Pradhan and Tang, but the success was limited as a diagnostic tool. More work is needed.

The samples were excited with the third harmonic 353 nm, 8ps pulse of a mode-locked Nd:glass laser with 30 μj energy. The fluorescence lifetimes were measured at different wavelengths using a streak camera with 10 ps resolution. The intensity profile displays double exponential fluorescence decay. It appears that the fast component of the decay in the malignant tumors is faster (less than 150 ps) than that of the benign tumors (greater than 200 ps). The slow component of the decay from malignant tumors is seen to be slower (greater than 2.4 ns) than that of the benign tumors (less than 2.4 ns). The faster fast

component of the fluorescence decay from malignant tumor implies more non-radiative processes existed in these samples. But no more conclusions can be reached yet.

Time-resolved fluorescence kinetics from normal, benign and cancerous tissues also were measured using a 60fs laser and a 2ps resolution streak camera. The excitation wavelength is at 310 nm that is the second harmonic of a CPM mode-locked dye laser. The fluorescence lifetimes were measured at the 340 nm emission wavelength. So far, 3 normal samples, 4 benign samples, and 4 normal samples were measured. The fast components of the fluorescence from normal, benign, and cancerous tissues are 86 ± 12 ps; 54 ± 6 ps; 77 ± 21 ps, respectively. The slow components of the fluorescence lifetimes from the three kinds of samples are 1.45 ± 0.14 ns; 1.00 ± 0.15 ns; 2.01 ± 0.05 ns, respectively. The ratios of the relative fluorescence intensities of fast components to that of slow components are 1.01 ± 0.24; 1.58 ± 0.60; 0.33 ± 0.1 for normal, benign, and cancerous tissues, respectively. One notices that the fast components of the fluorescence lifetime from these three kinds of samples are not very different. There may be slight differences of the slow components which are two times between benign and cancerous tissues, and the differences of the ratios are four times between the

both. These differences indicate that the time-resolved fluorescence method may not be useful tool to separate the normal and abnormal human tissues. But, more work is needed on larger number of samples.

(5) Fluorescence spectroscopy from cells

(a) Visible Radiation

Visible fluorescence spectra in the region from 500nm to 680nm, excited by argon laser beam at a wavelength of 488nm were measured from cultured human normal breast cells and three lines of cultured human breast cancer cells. The typical average spectra were from experiments on two to four samples. The results show that both the cancer and the normal cells display smooth fluorescence profiles with one main peak. The position of the peak was slightly shifted with a slight shape change between the normal cell spectral profile and the cancer cell spectral profiles, as well as between the different types of cancer cells .

The same type of experiments were carried out on human normal tissues, human cancer tissues, the interstitial media inside the normal breast tissues, and the left over part of the normal tissues after the

interstitial medium had been removed. This latter work was performed to determine the source of complex profile in fluorescence spectrum commonly observed from normal tissues in comparison to cancer tissues under visible excitation. The spectroscopic results showed that both the fresh normal tissue and the extracted interstitial media showed a three peak complex profile while the cancer tissues showed a single peak with smooth profile. The spectra from dried normal tissue (extracted interstitial media removed) showed an intent to change from a three peak complex profile to a smooth profile with single main peak. $NaCl_4Cl$ was added to normal tissues to extract blood. These spectral profiles became smooth with time in chemical environment.

The major peak at about 520nm may be attributed to the emission from the flavin molecular complex group. The experiments indicate that some molecular groups exist inside the interstitial media, such as hemoglobin/hemo proteins, of the normal tissue which are responsible to alter the spectral profiles excited by visible radiation of normal tissues as compared to the cancer tissues. This point was supported by theoretical calculation of fluorescence fluorescence including absorption from hemoglobins, and experiments using chemicals to remove hemoglobins.

(b) UV Excitation

The fluorescence spectra induced by UV excitation from cultured human breast normal cells and two lines of breast cancerous cells were measured. The cells were cultured with the same type of medium, under the same conditions, and using the same methods of preparation. Both the fluorescence and excitation spectra were measured from these different lines of cells. The profiles basically show a fluorescence band centered at about 340nm when the excitation wavelength was 300nm, and a fluorescence band centered about 450nm when the excitation was 320nm or 353nm.

Differences were found between the fluorescence spectra profile at the 450nm band from the normal and cancer breast cells when the excitation wavelength was 320nm and 353nm. There were also some differences between the excitation spectra from normal and cancer cells when the fluorescence wavelength was fixed at 460nm. The cancer cells showed relatively stronger excitation bands centered at around 330nm than the normal cells. The major emission bands in the fluorescence spectra from these cell samples may be attributed to the emission from some natural fluorophors which exist in the cells such as trytophan,

tyrosine, trypsin, NADH, NADPH, and the Y-base of t-RNA. The spectral differences indicated that either there may be environmental differences affecting the fluorophors inside the normal cells and cancer cells, and/or there may be different amounts of fluorophors present in these two types of samples. Much more work must be done to obtain a handle if consistent results are obtained.

(6) Light Scattering in Biological Media

Light scattering in biomedical media were studied using two novel techniques: time- and angle- resolved backscattering techniques. Our quantitative studies on temporal and angular profiles of the backscattered pulse can be used to determine the optical properties of biomedical media which in turn determine the state of the media. The temporal profiles of the backscattered pulse from various tissues are measured using a streak camera. Our experimental results on bovine eye shows that various stages of eye cataract can be determined from the temporal profile of the backscattered pulse. Backscattered pulse is also found to be able to differentiate fat plaque from the heart tissue, and lung from breast tissues. More importantly, our preliminary results indicate that the backscattered pulses from cancerous breast tissues differ from the

normal breast tissues.

The angular profile of the backscattered light from various biomedical tissues were measured and the intensity of the backscattered light is found to enhance around the backward direction. The optical properties can be determined from the angular profiles of the bacscattered light. These studies indicate that angular profile of the backscattered light may be a diagnostic tool in certain biomedical areas. Much more work is needed to determine its usefulness as a diagnostic approach.

II. List of Technical Reports

(1) Progress report #1	5/1/88
(2) Annual progress report	1/8/89
(3) Semi-Annual progress report	7/24/89
(4) Annual letter report	7/31/89
(5) Semi-Annual progress report	12/31/89
(6) Semi-Annual progress report	9/5/90
(7) Annual letter report	10/31/90
(8) Final technical report	1/31/91

IV. Publications and Patent Disclosures

(a) Publications

(1) "Optical Spectroscopy May Offer Diagnostic Approaches for Medical Profession." R. R. Alfano, A. Pradhan, G. C. Tang, B. B. Das, and K. M. Yoo, Submitted for publication in Leon Goldman's book "Laser Non-Surgical Medicine." Technomic Publishing Inc., to be published in May, 1991.

(2) "Ultra-Violet Fluorescence Spectra from Normal Tissues and Cancer Tumers of Gynecologogical Tract," W. Sha Glassman, C. H. Liu, G. C. Tang, and R. R. Alfano, Submitted to Lasers in Life Science, Janaury 24, 1991.

(3) "Near-IR Fourier Transform Raman Spectroscopy of Normal and atherosclerotic Human Aorta," C. H. Liu, W. L. sha, and R. R. Alfano; H. R. Zhu, and D. L. Akins; L. I. Deckelbaum, M. L. Stetz, K. O'Brien, and J. Scott; Submitted to Lasers in the Life Science, Janaury 22, 1991.

(4) "Human Breast Tissues Studied by IR Fourier Transform Raman Spectroscopy," R. R. Alfano, C. H. Liu, W. L. Sha; H. R. Zhu, D.

Akins; J. Cleary, R. Prudente, and E. Cellmer; Lasers in the Life Science, Vol.4, No1, 1-6, 1991.

(5) "Effects of Self-Absorption by Hemoglobins on the Fluorescence Spectra from Normal and Cancerous Tissues." C. H. Liu, G. C. Tang, Asima Pradhan, W. Sha and R. R. Alfano, Lasers in the Life Sciences, Vol.3(3) 167-176 1990.

(6) "Angle-and Time-resolved Studies of Backscattering of Light from Biological Tissues." K. M. Yoo, F. Liu, and R. R. Alfano, In Proceedings of SPIE: Time-resolved Laser Spectroscopy in Biochemistry II, Vol.1204, 492-498, 1990.

(7) "Coherent Backscattering of Light from Biological tissues." K. M. Yoo, G. C. Tang, and R. R. Alfano, Applied Optics, Vol.29, 3237, 1990.

(8) "Biological Material Probed by the Temporal and Angular Profiles of the Backscattered Ultrafast laser Pulses." K. M. Yoo, F. Liu, and R. R. Alfano, J.Opt.Soc.Am. B, Vol.7, 1685, 1990.

(9) "Determination of the Scattering and Absorption Lengths from the Temporal Profile of Backscattered Pulse." K. M. Yoo and R. R. Alfano, Optics Letters, Vol.15, 276,1990.

(10)"Time resolved backscattering of pulse to monitor different stages of eye cataract," R. A. Ahmed, K. M. Yoo, K. Klapper, and R. R. Alfano, Appl. Opt. 29, 896-897,1990. Appl. Phys. Lett. Vol.56, 1908, 1990.

(11)"When does the diffusion approximation fail to describe transport in random media," K. M. Yoo, F. Liu, and R. R. Alfano, Phys. Rev. Lett., Vol.64, 2647, 1990.

(12)"Light sheds light on cancer---distinguishing malignant tumors from benign tissues and tumors," R. R. Alfano, B. B. Das, J. Cleary, R. Prudente, and E. Celmer, Submitted to Bulletin of the New York Academy of Medicine, March 1990.

(13)"Spectroscopic Studies of Human Cancer and Normal Lung and Breast tissues." G.C.Tang, A.Pradham, R.R.Alfano, Lasers in Surgery and Medicine, Vol.9, 290-295, 1989.

(14)"Pulse and CW Laser Fluorescence Spectroscopy from Cancer and Chemically Treated Normal Breast and Lung Tissues." G. C. Tang, Asima Pradhan, Wenling Sha, J. Chen, C. H. Liu, S. J. Wahl and R. R. Alfano, Applied Optics, Vol.6, 1337-2437, 1989.

(15)"Optical Spectroscopic Diagnosis of Cancer and Normal Breast Tis-

sues." R. R. Alfano, Asima Pradhan and G. C. Tang, J. Opt. Soc. Am. B, Vol.6, No.5, 1015-1023, 1989. on Nov. 10, 1988.

(16)"Steady State and Time-Resolved Laser Fluorescence from Normal and Tumor Lung and Breast Tissues." R.R.Alfano, G.C.Tang, A.Pradhan and M.Bleich, D.S.J.Choy and E.Opher, Journal of Tumor Marker Oncology, Vol.3, 165-174,1988.

(17)"Fluorescence Spectra from Cancerous and Normal Human Breast and Lung Tissues." R.R.Alfano, G.C. Tang, A.Pradhan, W.Lam, D.S.J.Choy, E.Opher, IEEE QE-23, Vol.10, 1806-1811, 1987.

(b) Patent Disclosures

(1) Noninvasive characterization of media from the line shape of the coherent peak and the temporal profile of the scattered pulse Inventors: R. R. Alfano, K. M. Yoo, G. C. Tang Data witnessed: April 3, 1989, with MTC

(2) Method and apparatus of fluorescence spectroscopy to distinguish malignant tumors from benign tissues and tumors, and normal tissues Inventors R. R. Alfano, B. Das, G. C. Tang Date witnessed: October 13, 1989, with MTC

(3) Cancer--Diagnostic time resolved fluorescence instrumentation Inventors: R. R. Alfano, Asima Pradhan, G. C. Tang Date witnessed: June 13, 1990, with MTC

(4) IR Fourier Transform Raman Spectroscopy to determine state of human tissues Inventors: R. R. Alfano, C. H. Liu, W. L. Glassman Date witnessed: Augest 31, 1990, with MTC